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Effects of Ca^{2+} and Mg^{2+} on *Botrytis cinerea* and *Penicillium expansum in vitro* and on the biocontrol activity of *Candida oleophila*

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Calcium salts have been reported to play an important role in the inhibition of postharvest decay of apples and in enhancing the efficacy of postharvest biocontrol agents. Therefore, the present study was conducted in order to examine and compare the effects of calcium and magnesium salts on the germination and metabolism of the postharvest pathogens *Botrytis cinerea* and *Penicillium expansum*, and to determine the effects of these salts on the biocontrol activity of two isolates (182 and 247) of the yeast *Candida oleophila*. Increasing concentrations of $CaCl_2$ (25–175 mM) resulted in decreased spore germination and germ-tube growth of both pathogens. The greatest effect was observed in the case of *B. cinerea*. The inhibitory effect could be overcome by the addition of glucose to the germination medium. $MgCl_2$ (25–175 mM) had no effect on germination or germ-tube growth of either pathogen, indicating that the calcium cation rather than the chloride anion was responsible for the inhibition. The pectinolytic activity of crude enzyme obtained from the culture medium of both pathogens was also inhibited by 25–175 mM $CaCl_2$, with the greatest effect on the crude enzyme from *P. expansum*. Biocontrol activity of isolate 182 was enhanced by the addition of 90 or 180 mM $CaCl_2$, whereas there was no effect on the biocontrol activity of isolate 247. This was apparently due to the inability of isolate 247 to proliferate in apple wounds. It is postulated that enhanced biocontrol activity of isolate 182 of the yeast *C. oleophila* in the presence of Ca^{2+} ions is directly due to the inhibitory effects of calcium ions on pathogen spore germination and metabolism, and indirectly due to the ability of isolate 182 to maintain normal metabolism in the presence of 'toxic' levels of calcium.

INTRODUCTION

Biological control of postharvest diseases of fruits and vegetables is a new area of research (Wilson & Wisniewski, 1989; Droby *et al.*, 1991; Janisiewicz, 1991; Wisniewski & Wilson, 1992) which has emerged relatively recently as a promising alternative to the use of synthetic fungicides (Wilson & Wisniewski, 1994). The intensive research efforts that have been made to isolate, screen and test the efficacy of biocontrol agents under laboratory and semi-commercial conditions have resulted in the identification of a number of micro-organisms that protect fruits from infection. Research has been conducted on the biological control of postharvest diseases of apple, citrus, pome fruits and stone fruits, as well as other fruits and vegetables (Pusey & Wilson, 1984; Appel *et al.*, 1988; Janisiewicz, 1988; Chalutz & Wilson, 1989; Droby *et al.*, 1989; Wilson & Chalutz, 1989; Roberts, 1990; Droby *et al.*, 1991; Janisiewicz, 1991).

The efficacy of several postharvest biocontrol agents has been evaluated in pilot tests under semi-commercial conditions (Pusey *et al.*, 1988; Droby *et al.*, 1993; Hofstein *et al.*, 1993; Wisniewski *et al.*, 1993). In these tests, the use of antagonist preparations has conferred a level of protection similar to chemical fungicide treatment only when supplemented with reduced levels (5–10% of the recommended rate) of fungicide (Pusey *et al.*, 1988; Droby *et al.*, 1993). The results of pilot tests on citrus, utilizing the yeast antagonist *Pichia guilliermondii*, have indicated that a low concentration of the fungicide thiabendazole (TBZ) is needed in order to overcome the variability in the performance and to enhance the biocontrol activity of the yeast (Droby *et al.*, 1993). In order to replace

completely currently recommended synthetic fungicides with biocontrol agents, it will be neccessary to develop other methods to enhance their performance (Wilson & El Ghaouth, 1993).

Janisiewicz *et al.* (1992) demonstrated that, when using the antagonist *Pseudomonas syringae*, the addition of specific amino acids (L-asparagine and L-proline) enhanced biocontrol activity against the blue mould of apple caused by *Penicillium expansum*. It was suggested that the enhancement of *P. syringae* activity was due to the ability of the bacterium to outcompete the fungal pathogen for a specific nitrogen source, leading to enhanced growth of the bacterium in the wound site. Previous *in-vitro* screening of the nutritional requirements of the antagonist and the pathogen identified several amino acids that were selectively utilized by the antagonist and not by the pathogen. In an earlier study, McLaughlin *et al.* (1990) reported that the addition of 2% calcium chloride to a cell suspension of the yeast antagonist *P. guilliermondii* significantly enhanced its activity against postharvest diseases of apples. This allowed for a significant reduction in the amount of the yeast biomass required to achieve control.

Calcium salts have been reported to play an important role in the inhibition of postharvest decay of apples and potato. (Sharples & Johnson, 1977; Conway, 1982; McGuire & Kelman, 1986; Conway *et al.*, 1988). A similar effect of calcium, causing a decrease in the susceptibility of rose flowers (Volpin & Elad, 1991), *Ruscus* (Elad & Kirshner, 1992), beans and tomatoes (Elad & Volpin, 1993), and eggplant, pepper and cucumbers (Elad *et al.*, 1993) to the grey mould disease caused by *Botrytis cinerea* has been reported. However, the mode of action by which calcium inhibits disease development is not yet clearly understood. It has been suggested that calcium treatment increases the structural integrity of cell walls, via calcium crosslinking of pectins, enhancing their resistance to maceration by cell-wall-degrading enzymes of fungal origin (Conway *et al.*, 1988). However, a detrimental effect of high concentrations of calcium on fungal metabolism cannot be ruled out.

Preliminary research indicated that the addition of 2% calcium chloride to the formulation of the yeast biocontrol agent *Candida oleophila* enhanced the ability of this yeast to protect apples against postharvest decay (Wisniewski *et al.*, 1993). Therefore, it was of interest to examine further the direct and indirect effects of calcium and magnesium salts on the postharvest pathogens *B. cinerea* and *P. expansum*. The objective of the present study was to investigate the effects of calcium chloride on spore germination and growth of *B. cinerea* and *P. expansum*, the cause of grey and blue mould of apple, respectively, as well as the effects on the *in-vitro* activity of macerating enzymes produced by these fungal pathogens. In addition, the effect of calcium chloride on the biocontrol activity of two isolates of the yeast *C. oleophila* was examined.

MATERIALS AND METHODS

Antagonist and fungal cultures

Yeast strain 182 of *Candida oleophila* was isolated from the surface of tomato fruit as described by Wilson *et al.* (1993). Strain 247 of *C. oleophila* (ATCC 20372) was obtained from the American Type Culture Collection, Rockville, MD, USA. The yeast cultures were maintained at 4°C on Nutrient Yeast Dextrose Agar (NYDA) medium containing 8 g nutrient broth, 5 g yeast extract, 10 g glucose and 20 g agar, in 1 l of distilled water. Liquid cultures of the yeasts were grown in 100-ml Erlenmeyer flasks containing 25 ml of NYD Broth (NYDB) and inoculated with a loop of the culture. Flasks were incubated on a rotary shaker at 26°C for 48 h. Following incubation, cells were centrifuged at 7000 rpm and washed twice in order to remove the growth medium. Cell pellets were re-suspended in distilled sterilized water and brought to the initial concentration of 2×10^9 to 5 10^9 cells/ml. Cell concentration was then adjusted as necessary for the different experiments.

Cultures of *B. cinerea* and *P. expansum* were isolated from decayed apple fruit. They were maintained on potato dextrose agar (PDA) slants at 4°C, and fresh cultures were grown on PDA plates before use.

Pectinase assay

In order to obtain a crude pectinase preparation, PDA discs (5 mm in diameter) were cut with a cork borer from the growing edges of 1-week-old cultures of either *B. cinerea* or *P. expansum* and used to inoculate liquid yeast nitrogen base medium (BBL, Cockeysville, MD, USA) with 0·1 g/l apple pectin (Sigma Chemical Co., St Louis, MO, USA) as the carbon source. Five PDA discs were added to each 150-ml Erlenmeyer flask containing 50 ml of growth medium.

Following 1 week of incubation on a rotary shaker, fungal cultures were precipitated by centrifugation at 7000 rpm and the culture medium was filtered using a 0·22-μm filter. The culture filtrate was used to measure enzymatic activity. Pectinase activity was determined using the dinitrosalicylate assay (Miller, 1959) to measure the rate of release of reducing sugars spectrophotometrically. The reaction mixture contained 0·5 ml of substrate (0·1 g/l apple pectin in 0·1 M acetate buffer, pH 4·2), 0·0–175 mM $CaCl_2$, and 0·5 ml of crude enzyme preparation. The mixture was incubated for 5 h at 37°C and reactions were terminated by the addition of 3 ml of DNS reagent (dinitrosalicylic acid, 1%; phenol, 0·2%; sodium sulphite, 0·05% dissolved in a 1% NaOH solution). The reaction mixture was then brought up to a final volume of 5 ml by adding 1 ml of distilled water and immersed in a boiling water bath for 15 min. The amount of reducing sugars was determined with a spectrophotometer at 575 nm following equilibration of the solutions to 25°C. Reaction mixtures containing boiled enzyme preparations or reactions terminated at zero time were used as controls. Activity was expressed as the amount of reducing sugars (μmol) released per hour at 37°C. D-galactose was used as a standard. Three replicates were used for each treatment and the experiment was repeated three times. The results of all the experiments were similar, and the data from a single experiment are illustrated.

Spore germination and growth *in vitro*

Spore suspensions were prepared from 1 to 2-week-old cultures of *B. cinerea* or *P. expansum*. The spores were removed from the surface of the cultures using a sterile bacteriological loop, suspended in 10 ml of sterile distilled water, and filtered through four layers of sterile cheesecloth in order to remove any adhering mycelia. The spore concentration was adjusted to 10^6 spores/ml with a haemocytometer. Aliquots of a spore suspension of either *B. cinerea* or *P. expansum* were added to wells of a 96-well microtitration plate containing various concentrations of sugar and divalent ions ($CaCl_2$, $MgCl_2$) to give a final concentration of 10^5 fungal spores/ml. After incubation for 24 h at room temperature, glutaraldehyde was added to each well to give a final concentration of 5%. Wells were then assayed in order to determine percentage spore germination and germ-tube length using an inverted microscope, video camera and JAVA image analysis software (Jandel Scientific, Corte Madera, CA, USA). Each experiment was replicated three times, and at least 100 spores and 40 germ tubes were viewed for each replicate.

Yeast population in apple wounds

To determine the effect of $CaCl_2$ and $MgCl_2$ on the population of two isolates (182 and 247) of *C. oleophila* in apple wounds, 20 μl of a water suspension of yeast cells (10^6 cells/ml) containing various concentrations of $CaCl_2$ or $MgCl_2$ were pipetted into each wound and incubated at ambient temperatures under moist conditions in plastic food trays with lids. Uniform wounds (2·0 mm in diameter and 4·0 mm deep) were made with a small nail. Tissue containing the wound was removed with a cork borer (10·0 mm in diameter) at 0, 5, 24 and 48 h and homogenized in 5 ml of sterile water. Samples were serially diluted and plated on potato dextrose agar (PDA) in order to determine the yeast cell concentration. Three wounds were inoculated for each treatment, and the average of three PDA plates was used to determine the population of yeast for each wound. The results are expressed as the mean number (± SEM) of colony-forming units (CFU) per wound ($n = 3$).

Biocontrol activity of yeast antagonists

To test the biocontrol efficacy of two strains (182 and 247) of the yeast *C. oleophila*, apples (cv. 'Golden Delicious') were washed with tap water, dried, and a uniform wound 4 mm deep and 2–3 mm in diameter was made on the side of each fruit. Wounds were inoculated with 40 μl of yeast suspension in various concentrations of $CaCl_2$. After 3 h, 20 μl of sterile water containing either 10^4 spores of *Penicillium expansum* or 10^5 spores of *Botrytis cinerea* were added to each wound. Treated fruits were stored at ambient temperature and high relative humidity in plastic food trays with lids. Lesion diameter was recorded 10 days after inoculation. Each treatment consisted of nine fruits, with one wound per fruit.

RESULTS

Effect of $CaCl_2$ and $MgCl_2$ on spore germination and growth

The effect of 25–175 mM $CaCl_2$ or $MgCl_2$, with or without the addition of 0·1–5·0 mM glucose, on spore germination of *B. cinerea* and *P. expansum* is shown in Fig. 1. Increasing

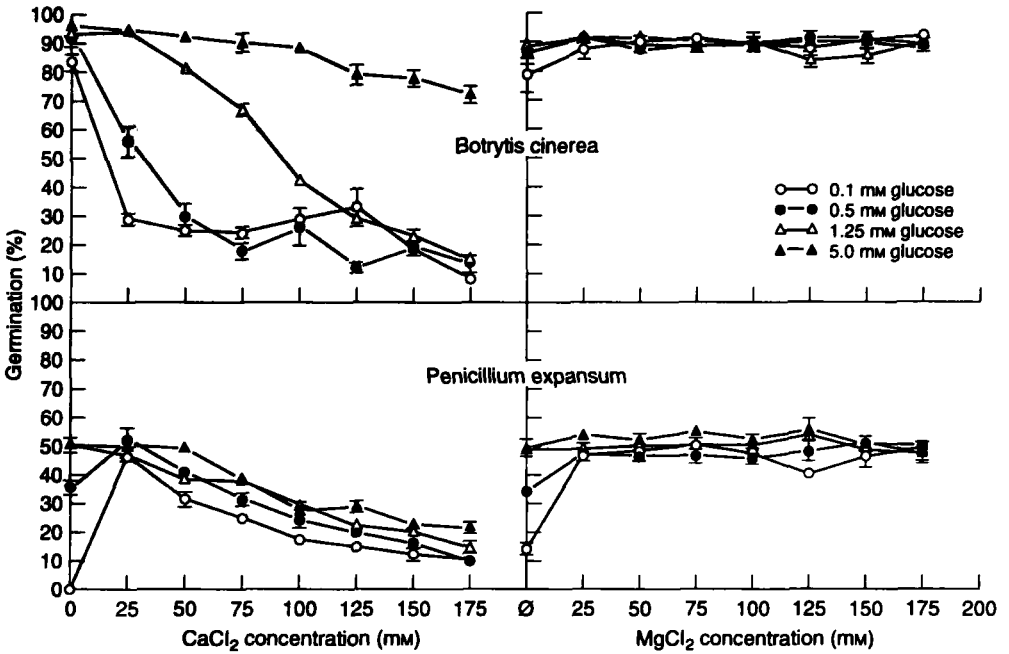

Fig. 1 Effect of 25·0–175 mM $CaCl_2$ or $MgCl_2$ on percentage germination of *B. cinerea* and *P. expansum* in the presence of 0·1–5·0 mM glucose. Mean values ± SEM are shown ($n = 3$). At least 100 spores were measured for each treatment within each replicate.

concentrations of $CaCl_2$ significantly affected spore germination of both fungal pathogens at all glucose concentrations. However, the inhibitory effect of the $CaCl_2$ was reduced in the presence of increasing levels of glucose. This was especially evident in the germination of *B. cinerea* spores in the presence of 5 mM glucose.

Spore germination of *P. expansum* was not as greatly affected by the addition of $CaCl_2$ as was that of *B. cinerea* (Fig. 1). Percentage spore germination was reduced from about 50% in controls and in the presence of 25 mM $CaCl_2$ to 10–30% at $CaCl_2$ concentrations higher than 50 mM. Although it appeared that glucose stimulated spore germination in the absence of $CaCl_2$, only a small change was observed in the ability of higher concentrations of glucose to reduce the inhibitory effect of $CaCl_2$. In contrast to the situation with $CaCl_2$, no consistent effect on spore germination of either fungal pathogen was observed with the addition of 25–175 mM $MgCl_2$.

As is shown in Fig. 2, germ-tube elongation of both fungal pathogens was also inhibited by increasing concentrations of $CaCl_2$ (20–175 mM). As in the case of spore germination, the inhibitory effect of $CaCl_2$ was more readily apparent with *B. cinerea* than with *P. expansum*. Increasing levels of glucose also reduced the inhibitory effect of $CaCl_2$. Overall, however, the concentration of glucose required to reduce the inhibitory effect of $CaCl_2$ was much greater for germ-tube elongation (5·0–60 mM glucose) than for spore germination (0·5–5·0 mM glucose). No inhibitory effect on germ-tube elongation was observed when $MgCl_2$ was used.

Growth of yeast in apple wounds

The population dynamics of isolates 182 and 247 of *C. oleophila* were determined in apple wounds in the presence of 180 mM $CaCl_2$ or 201 mM $MgCl_2$. Yeast cell counts of isolate 182, expressed as the number of colony-forming units per wound, were not affected by the addition of either $CaCl_2$ or $MgCl_2$. An increase in the population in the wounds by about two orders of magnitude was evident following incubation for 24 and 48 h, regardless of the salt added (Fig. 3). The number of cells increased rapidly, from $5{\cdot}0 \times 10^5$ to approximately $1{\cdot}0 \times 10^8$ within 24 h. In contrast, the population of isolate 247 did not increase to the same extent as that of

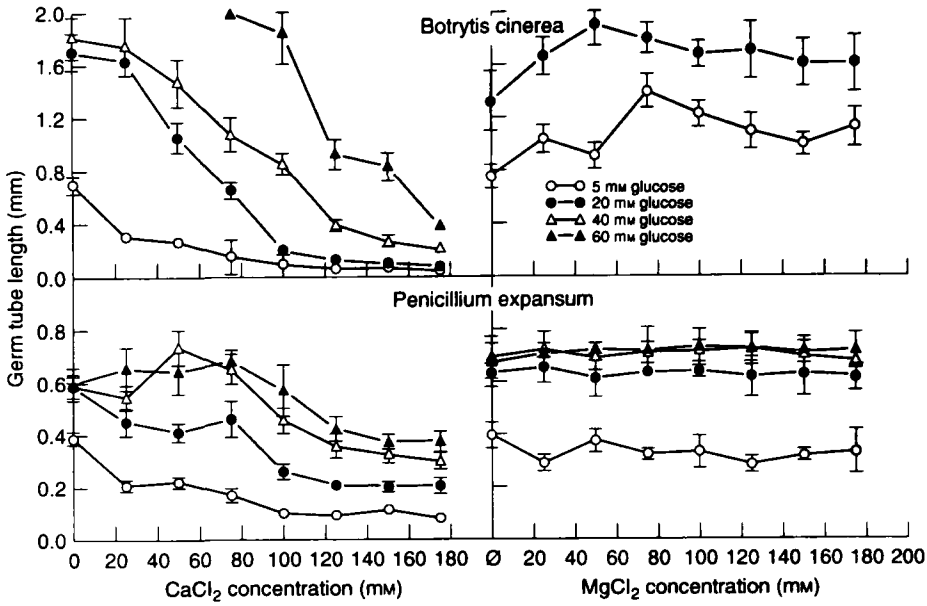

Fig. 2 Effect of 25·0–175 mM $CaCl_2$ or $MgCl_2$ on germ-tube length of *B. cinerea* and *P. expansum* in the presence of 5·0–60 mM glucose. Mean values ± SEM are shown ($n = 3$). At least 40 germ tubes were measured for each treatment within each replicate.

isolate 182. The population of isolate 247 did not increase above 7 10^6 CFU wound, even though no discernible effect on growth was observed after the addition of $CaCl_2$ or $MgCl_2$.

Enhancement of biocontrol activity with $CaCl_2$

The effect of the application of 90 and 180 mM $CaCl_2$ to apple wounds, with and without the

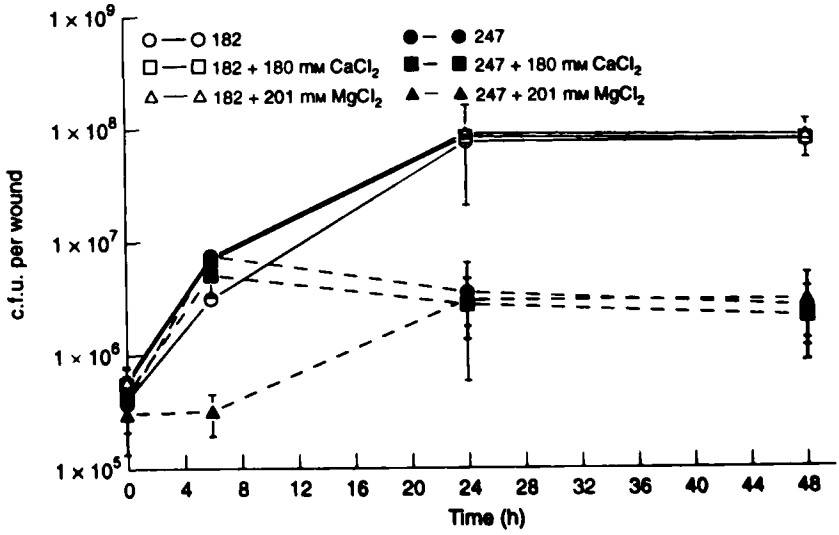

Fig. 3 Growth of isolates 182 and 247 of *Candida oleophila* in apple wounds in the presence of 180 mM $CaCl_2$ or 201 mM $MgCl_2$. Results are expressed as mean numbers of colony-forming units (CFU) per wound. Mean values ± SEM are shown ($n = 3$).

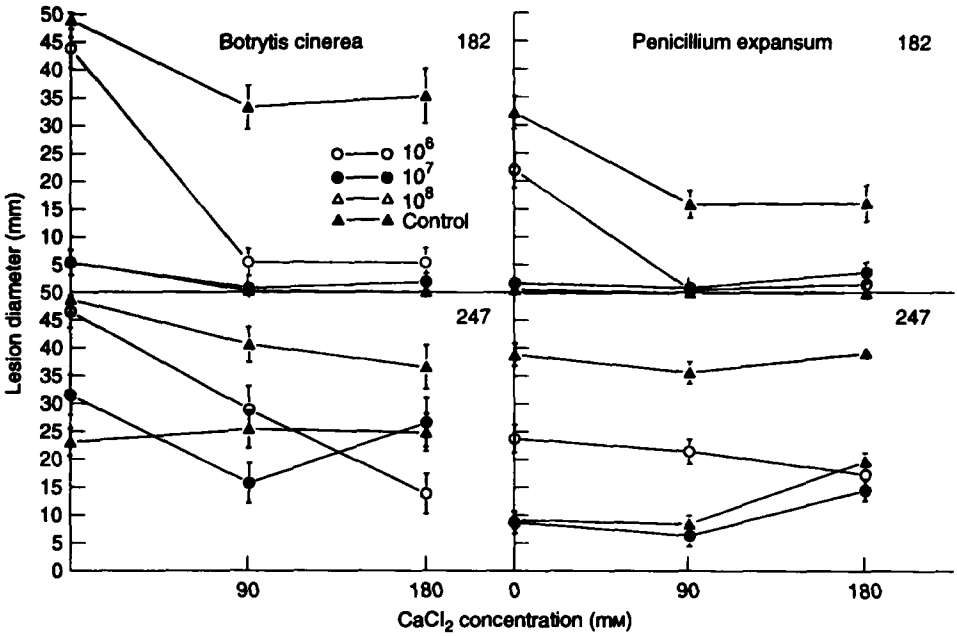

Fig. 4 Effect of 90 or 180 mM $CaCl_2$ on biocontrol activity of two isolates (182 and 247) of *C. oleophila* against *B cinerea* and *P. expansum* on apple. Results are expressed as mean lesion diameter ± SEM (n = 9). Lesion diameter was measured 10 days after inoculation with the pathogens and yeast.

addition of yeast isolates 182 and 247, on infection development by *B. cinerea* and *P. expansum* is shown in Fig. 4. The results indicate that treatment of apple wounds with $CaCl_2$ alone (control) resulted in only a slight reduction in lesion diameter, as measured after 10 days of incubation at ambient temperatures (20–22°C). However, the addition of $CaCl_2$, at both concentrations tested, combined with a cell suspension of isolate 182 of *C. oleophila*, markedly enhanced the efficacy of this biocontrol agent against decay development by either *B. cinerea* or *P. expansum*. When 10^7 or 10^8 cells/ml of isolate 182 were applied to inoculated wounds directly, with or without $CaCl_2$, development of lesions was limited to a diameter ranging from 2 to 5 mm in apples inoculated with either pathogen. In contrast, the lesion diameter in the control fruit inoculated with either *B. cinerea* or *P. expansum* was 48 and 33 mm, respectively. Application of isolate 182 alone at 10^6 cells/ml resulted in a negligible reduction in lesion diameter. However, lesion diameter was reduced from 45 mm to 5 mm when isolate 182 at 10^6 cells/ml, supplemented with either 90 or 180 mM $CaCl_2$, was applied to the wounds prior to inoculation with either pathogen.

Isolate 247 of *C. oleophila* was not as effective as isolate 182 in preventing infection of apple wounds by *B. cinerea* and *P. expansum* (Fig. 4). Furthermore, the interaction of this isolate with $CaCl_2$ was not similar to that of isolate 182. The addition of $CaCl_2$ to cell suspensions of isolate 247 failed to inhibit lesion development by either pathogen, compared to control wounds treated with yeast cells alone. Instead, a small increase in lesion diameter by both *B. cinerea* and *P. expansum* was observed when the yeast cell suspensions (10^7 and 10^8 cells/ml) were supplemented with 180 mM $CaCl_2$. An effect of $CaCl_2$ on biocontrol activity of isolate 247 was only observed against *B. cinerea* when 90 and 180 mM of $CaCl_2$ were added to a cell suspension of 10^6 cells/ml. Lesion diameter was reduced from 44 mm in control wounds which were treated with yeast cells alone to 30 and 15 mm in the presence of 90 and 180 mM $CaCl_2$, respectively (Fig. 4). The reason for this exception was not clear.

Effect of $CaCl_2$ on pectinolytic activity *in vitro*

The effect of $CaCl_2$ on pectinolytic activity, measured as the amount of reducing sugars

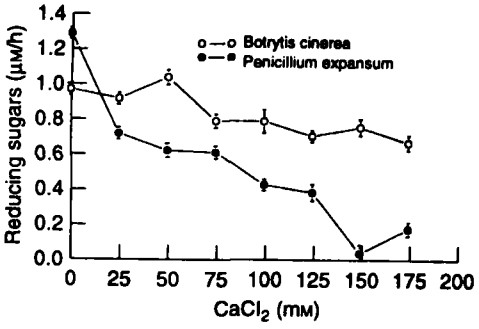

Fig. 5 Effect of 25·0–175 mM $CaCl_2$ on pectinolytic activity of crude enzyme obtained from *B. cinerea* and *P. expansum* culture medium. Mean values ± SEM are shown ($n = 3$). The experiment was repeated three times.

released from a pectin substrate by crude enzyme preparations obtained from *B. cinerea* or *P. expansum*, is shown in Fig. 5. The ability of the *B. cinerea* crude enzyme to hydrolyse the pectin substrate was not affected by the addition of increasing concentrations of $CaCl_2$ to the same degree as the crude enzyme preparation obtained from *P. expansum*. Although all $CaCl_2$ concentrations tested (25–175 mM) inhibited the release of reducing sugars from the pectin substrate, the greatest effect was on the enzyme preparation derived from *P. expansum*. Intermediate inhibition of pectinase obtained from *P. expansum* was observed when $CaCl_2$ was added to the reaction mixture at concentrations of 25, 50 or 75 mM. Strong inhibition of pectinase activity was observed when the concentration of $CaCl_2$ was increased to 125, 150 or 175 mM.

DISCUSSION

The results of this study demonstrate the beneficial effect of calcium on the biocontrol activity of isolate 182 of *C. oleophila*. The inclusion of 90 mM $CaCl_2$ with a cell suspension of isolate 182 resulted in a marked improvement in antagonistic activity when the isolate was applied to apple wounds at a concentration of 10^6 cells/ml (Fig.4). The same concentration of yeast cells without calcium failed to protect wounds against the development of *B. cinerea* or *P. expansum*. Such beneficial effects on biocontrol activity, achieved by the addition of calcium, were also observed by McLaughlin *et al.* (1990) using the yeast *Pichia guilliermondii* as a postharvest biocontrol agent. A wide range of salts was tested in that study and calcium was found to be the most effective cation, whereas magnesium had no effect. Another study in which magnesium, calcium or strontium chloride solutions were pressure-infiltrated into apples also indicated that calcium was the most effective cation, whereas magnesium was injurious to apple tissue (Conway & Sams, 1987).

The effect of calcium may have important implications for the future use of yeast on a commercial scale for the control of postharvest diseases of fruit. Calcium, as part of the formulation for these biocontrol agents, could be used to increase efficacy, thereby replacing the current requirement for addition of low concentrations of fungicides to ensure consistent performance of yeast biocontrol agents under large-scale and commercial conditions (Droby *et al.*, 1993). As reported by Droby *et al.* (1989), effective biocontrol activity of yeast antagonists depends on the number of yeast cells present in the wound site. The addition of calcium would increase the ability of the yeast to inhibit the development of the pathogen by lowering the concentration of yeast required for effective biocontrol.

The application of $CaCl_2$ with isolate 247 of *C. oleophila* did not generally result in any improvement in the ability of this yeast to inhibit either pathogen, even when the yeast was applied to apple wounds at high concentrations (10^7 and 10^8 cells/ml). Since there was no discernible effect of calcium on the growth of either isolate, the lack of biocontrol can most probably be attributed to the inability of isolate 247 to develop and maintain a high population in the wound site (Fig. 3).

Our results indicate that calcium may reduce fungal infection through direct inhibition of spore germination and growth, as well as through an effect on the activity of cell-wall-macerating enzymes (Figs 1, 2 and 5). The present study also indicates that calcium has a greater effect on the processes of spore germination and growth of *B. cinerea* than of *P. expansum*, whereas the opposite was true for the effect of calcium on pectinolytic enzymes. Calcium decreased pectinolytic activity by over 90% in *P. expansum* and by only 20% in *B. cinerea*. Although these results are based on *in-vitro* assays alone, similar inhibitory activity could occur at the wound site, where free calcium ions might be present. However, the precise mechanism of the inhibition of fungal spore germination and germ-tube elongation is not yet understood.

The inhibition, *in vitro*, of *P. expansum*-derived pectinase by calcium could be caused by reduced availability of the substrate for hydrolysis, due to changes in pectin configuration. Previous studies have indicated that an increase in the calcium content of cell walls of apples reduces the activity of polygalacturonase extracted from *P. expansum* cultures (Conway *et al.*, 1988). McGuire & Kelman (1986) also demonstrated a relationship between increasing levels of calcium in the cell walls of potato tubers and a reduction in the macerating activity of *Erwinia carotovora*. The effect of calcium salts on various processes of fruit ripening, such as a delay in senescence, a reduction in physiological disorders (Ferguson, 1984; Klein *et al.*, 1990), and a reduction in the susceptibility of fruit to the development of postharvest decay, have already been reported (Conway, 1982; Conway *et al.*, 1988).

Our results also indicate that there is no inhibitory effect of $MgCl_2$ on the growth of either isolate of *C. oleophila*. Furthermore, there was no discernible effect of magnesium on spore germination or germ-tube growth of either pathogen. Previous work (McLaughlin *et al.*, 1990) has demonstrated that $MgCl_2$ does not enhance biocontrol activity of the yeast *Candida guilliermondi*. Similar results were obtained in the present study when $MgCl_2$ was added to the yeast formulation prior to the application to wounded apples (data not shown). This would suggest that Ca^{2+} cations, rather than chloride anions, are essential for enhancing the biocontrol activity of yeast antagonists. Why the calcium cation should be more effective than the magnesium cation remains an open question. Both of the divalent cations are capable of crosslinking pectin, which in turn would render the pectin less susceptible to enzymatic degradation by the fungal enzymes. However, high levels of calcium may be more detrimental to metabolism, as evidenced by the inhibitory effects on spore germination and germ-tube growth, because of the integral role in so many biochemical and signalling pathways (Poovaiah & Reddy, 1987; Rasmussen & Rasmussen, 1990).

The addition of increasing concentrations of glucose partially reversed the inhibitory effect of calcium on germination and germ-tube elongation of *B. cinerea* and *P. expansum*. This indicates that the level of carbon source available for energy utilization is related to the ability of the pathogen to overcome the detrimental effects of high concentrations of calcium. In higher eukaryotic cells, high levels of internal calcium are known to cause inhibition of growth, and may lead to cell death (Carafoli, 1987). In addition, high concentrations of extracellular calcium in conjuction with a shortage of ATP lead to elevated levels of internal calcium in yeast cells (Halachmi & Eilam, 1989).

The present study has shown that, although the pathogens *B. cinerea* and *P. expansum* were inhibited by increasing concentrations of Ca^{2+} ions, growth of the yeast isolate 182 of *C. oleophila* was not affected. This suggests that there are inherent differences between the yeast and the pathogen in their sensitivity to calcium and in the mechanism of calcium transport. These differences would determine the level of exogenous calcium that would either be beneficial to or have no effect on the yeast, while having a deleterious effect on the pathogen. Therefore we postulate that enhanced biocontrol activity by isolate 182 of *C. oleophila* in the presence of Ca^{2+} ions is directly due to the inhibitory effects of calcium ions on pathogen germination and metabolism and indirectly due to the ability of isolate 182 to maintain normal metabolism in the presence of 'toxic' levels of calcium.

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